ELSEVIER

European Journal of Pharmacology 309 (1996) 251–259

ejp

Analysis of responses to angiotensin I and angiotensin I-(3–10) in the mesenteric vascular bed of the cat

Hunter C. Champion, Etoi A. Garrison, Lance S. Estrada, Jeffery M. Potter, Philip J. Kadowitz *

Department of Pharmacology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, USA

Received 11 April 1996; accepted 7 May 1996

Abstract

Responses to angiotensin I and antiogensin I-(3–10), the precursors for angiotensin II and IV, were investigated in the mesenteric vascular bed of the cat. Under constant-flow conditions, injections of precursors and the active peptides into the mesenteric arterial perfusion circuit caused dose-related increases in receptor antagonist that were attenuated by the angiotensin AT_1 receptor antagonist DuP532 (2-propyl-4-pentafluoroethyl-1-[2′-(2 *H*-tetrazol-5-YL)-1,1′-biphenyl-4-YL methyl]1 *H*-imidazole-5-carboxylic acid), but not by the angiotensin AT_2 receptor antagonist PD123,319 ((*S*)1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1 *H*-imadazo[4,5-c]pyridine-6-carboxylic acid, ditriflouroacetate]). Responses to angiotensin I and II were similar as were responses to angiotensin I-(3–10) and angiotensin IV, and these responses were not altered by the presence of a time-delay coil in the perfusion circuit. Responses to angiotensin I and angiotensin I-(3–10) were decreased by the angiotensin converting enzyme inhibitor enalaprilat in a dose of the angiotensin converting enzyme inhibitor that had no effect on responses to angiotensin II and IV and that enhanced vasodilator responses to bradykinin. The putative angiotensin AT_2 receptor agonist, *p*-aminophenylalanine^6-angiotensin II, produced dose-related increases in mesenteric arterial perfusion pressure that were reduced by DUP532, suggesting that they are mediated by angiotensin AT_1 receptors. These results suggest that angiotensin I and angiotensin I-(3–10) are rapidly and efficiently converted by an angiotensin converting enzyme-dependent pathway into active peptides that induce vasoconstriction by activating angiotensin AT_1 receptors in the mesenteric vascular bed of the cat.

Keywords: Intestinal vascular bed; Angiotensin peptide; DuP532; PD123,319; Enalaprilat; Angiotensin II; Angiotensin IV; *p*-Aminophenylalanine^6-angiotensin II

1. Introduction

Angiotensin II is a potent vasoactive peptide that plays an important role in the regulation of vasomotor tone, sodium and water homeostasis (Regoli et al., 1974; Peach, 1977; Timmermans et al., 1993). Responses to angiotensin II are mediated by specific cell surface receptors, and the development of potent non-peptide angiotensin II receptor antagonists has led to the identification of two major angiotensin II receptor subtypes, AT_1 and AT_2 (Blankley et al., 1991; Timmermans et al., 1993). It is generally accepted that most, if not all, of the cardiovascular responses to angiotensin II are mediated by angiotensin AT_1 receptor activation (Bottari et al., 1993; Timmermans et al., 1993). Less is known regarding the functional importance of the angiotensin AT_2 receptor, and it has been suggested that angiotensin AT_2 receptor activation may play a role in the regulation of fetal growth and development (Bottari et al., 1993).

The primary site for the conversion of angiotensin I to angiotensin II is historically believed to occur in the lung where the angiotensin converting enzyme is located on the surface of pulmonary capillary endothelial cells (Peach, 1977). Recent studies have suggested that, in addition to the pulmonary capillary bed, there may be significant angiotensin converting enzyme activity in the upstream resistance vessel segments within the hindlimb and pulmonary vascular beds of the cat (Cheng et al., 1994; Garrison et al., 1995). Angiotensin III may be formed from angiotensin II by aminopeptidase A, and the heptapeptide has been reported to be equipotent to angiotensin II in eliciting vasoconstriction and stimulating the release of aldosterone from the adrenal cortex (Semple et al., 1976;

* Corresponding author. Tel.: +1 504 588 5444; fax: +1 504 5888 5283.

PII S0014-2999(96)00355-X

Cheng et al., 1994; Nossaman et al., 1994; Chiu et al., 1976).

Angiotensin IV, a hexapeptide, is historically believed to have little if any biological activity (Blair-West et al., 1971; Harding et al., 1992; Bernier et al., 1994). Angiotensin IV may be formed from angiotensin II by aminopeptidase M, or alternatively, by an angiotensin converting enzyme-dependent pathway from the precursor peptide angiotensin I-(3–10) (Blair-West et al., 1971; Semple et al., 1976; Ward, 1984; Ahmad and Ward, 1990). Recently, angiotensin IV was reported to exhibit vasodilator activity in rabbit brain arterioles when administered with L-arginine and to increase renal cortical blood flow when infused in the anesthetized rat (Haberl et al., 1991; Swanson et al., 1992). In normal human subjects, angiotensin IV is reported to increase mean arterial pressure (Kono et al., 1985) and has been shown to produce dose-dependent reductions in renal and mesenteric blood flow in the conscious rat (Gardiner et al., 1993). Although angiotensin IV has been shown to possess significant vasoconstrictor activity in the hindlimb and pulmonary vascular beds of the cat, little if anything is known about responses to angiotensin IV or its precursor angiotensin I-(3–10) in the mesenteric vascular bed (Cheng et al., 1994; Garrison et al., 1995).

The present study was, therefore, undertaken to investigate responses to angiotensin IV and its precursor, angiotensin I-(3–10), and to compare these responses with those elicited by angiotensin II and its precursor, angiotensin I, in the mesenteric vascular bed of the cat. The receptor subtype mediating responses to the angiotensin peptides and the putative angiotensin AT_2 receptor agonist, *p*-aminophenylalanine^6-angiotensin II, were investigated using the angiotensin AT_1 receptor antagonist, DuP532 (2-propyl-4-pentafluoroethyl-1-[2′-(2*H*-tetrazol-5-YL)-1,1′-biphenyl-4-YL methyl]1*H*-imidazole-5-carboxylic acid), and the angiotension AT_2 receptor antagonist, PD123,319 ((*S*)1-[[4-(dimethylamino)-3-methylphenyl]-methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imadazo-[4,5-c]pyridine-6-carboxylic acid, ditriflouroacetate]). The role of angiotensin converting enzyme in mediating responses to angiotensin I-(3–10) and angiotensin I in the mesenteric vascular bed were investigated using the angiotensin converting enzyme inhibitor, enalaprilat.

2. Materials and methods

Fifty-two adult cats unselected as to sex, weighing 2.5–4.0 kg, were sedated with ketamine (10–15 mg/kg i.m.) and anesthetized with pentobarbital sodium (30 mg/kg i.v.). Supplemental doses of pentobarbital were given as needed to maintain a uniform level of anesthesia. The trachea was cannulated to ensure a patent airway, and the animals breathed room air or were ventilated with a Harvard model 607 respiratory at a tidal volume of 40–60 ml at a rate of 15–22 breaths/min. Catheters were inserted into the external jugular vein for the i.v. administration of drugs and into the carotid artery for the measurement of systemic arterial (aortic) pressure. For constant-flow perfusion of the mesenteric vascular bed, the superior mesenteric artery was approached through a midline abdominal incision and cleared of surrounding connective tissue. Following the administration of heparin sodium (1500 U/kg), the femoral artery was cannulated and connected to the inlet side of the perfusion circuit. The outlet side of the perfusion circuit was connected to a catheter inserted into the superior mesenteric artery. Blood flow to the small intestine was maintained constant with a Sigmamotor model T-8 peristaltic pump.

Superior mesenteric arterial perfusion pressure was measured by way of a lateral tap in the perfusion circuit located between the pump and the outlet side of the perfusion circuit. Superior mesenteric arterial perfusion pressure and systemic arterial pressure were measured with Statham P23 pressure transducers and were recorded on a Grass model 7 polygraph. Mean pressures were derived from the pulsatile pressure signal by electronic averaging, and the perfusion rate was set so that superior mesenteric arterial perfusion pressure approximated systematic arterial pressure and was not changed during an experiment. The flow rate was determined by timed collection and ranged from 25 to 32 ml/min. The agonists used in these experiments were injected directly into the superior mesenteric arterial perfusion circuit distal to the pump in small volumes (30 and 100 μl), and the superior mesenteric ganglion was ligated and cut to denervate the superior mesenteric vascular bed. These procedures were described previously (Osei et al., 1993).

In the present study, five series of experiments were performed; and in the first series of experiments, responses to angiotensin, I, II, IV, and angiotensin I-(3–10) were compared on a nmol basis. The dose range for the angiotensin peptides was found to be 0.01–100 μg in pilot experiments; and responses to the angiotensin peptides were reproducible with respect to time; and tachyphylaxis was not observed when injections were repeated at 10–20 min intervals. In the second series of experiments the effect of a 30–60 s time-delay coil on the inlet side of the perfusion circuit on the response to angiotensin I and angiotensin I-(3–10) was investigated to ascertain if peripheral conversion and recirculation of active peptide contributed to the increase in mesenteric arterial perfusion in response to the precursors. The time-course of the response of the increase in mesenteric arterial perfusion pressure was assessed by measuring and plotting perfusion pressure at 10 s intervals. In the third series of experiments the effect of the angiotensin converting enzyme inhibitor, enalaprilat, in a dose of 4 mg/kg i.v. on responses to angiotensin I and angiotensin I-(3–10) were investigated. In these experiments the effects of the angiotensin converting enzyme inhibitor on responses to bradykinin, norepi-

nephrine, angiotensin II, and angiotensin IV were investigated. In the fourth and fifth series of experiments, the effects of the angiotensin II subtype 1 (AT_1) receptor antagonist DuP532 (100 μg/kg i.v.) and the angiotensin II subtype 2 (AT_2) receptor antagonist PD123,319 (5–20 mg/kg i.v.) on responses to the angiotensin peptides and to norepinephrine were investigated. In the last series of experiments responses to the putative angiotensin AT_2 receptor agonist, *p*-aminophenylalanine^6-angiotensin II, were investigated; and the effects of DUP532 and PD123,319 on responses to *p*-aminophenylalanine^6-angiotensin II were ascertained.

The agonists used in these studies were norepinephrine bitarrate, angiotensin I, angiotensin II, *p*-aminophenylalanine^6-angiotensin II (Sigma Chemical, St. Louis, MO, USA), and angiotensin IV (Peninsula, Belmont, CA, USA) and were dissolved in 0.9% NaCl. Angiotensin I-(3–10) was prepared by Ms. Carol G. Carlton in the Department of Biochemistry at Tulane University School of Medicine on an Applied Biosystems model 430A peptide synthesizer using solid-phase synthesis with Fast-Moc chemistry. The purity of the angiotensin I-(3–10) was assessed by mass spectroscopy using a Vastec 201 mass spectrometer and an Electrospray interface with a quadrupole mass analyzer and was estimated to be > 99%. All agonists were injected directly into the superior mesenteric arterial perfusion circuit. The antagonists or inhibitors used in these studies were DuP532 (2-propyl-4-pentafluoroethyl-1-[2′-(2*H*-tetrazol-5-YL)-1,1′-biphenyl-4-YL methyl]1*H*-imidazole-5-carboxylic acid; DuPont-Merck, Wilington, DE, USA), PD 123319 ((*S*)1-[[4-(dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imadazo[4,5-c]pyridine-6-carboxylic acid, ditriflouroacetate]; Parke-Davis, Ann Arbor, MI, USA), and enalaprilat (Merck Sharp and Dohme, West Point, PA, USA) and were dissolved in 0.9% NaCl and administered intravenously. Responses to the agonists used in these studies were compared before and beginning 10 min after administration of the angiotensin converting enzyme inhibitor or the angiotensin receptor blockers. All drug solutions were prepared on a frequent basis, stored in brown stoppered bottles, and kept on crushed ice during an experiment.

The hemodynamic data are expresed as mean ± S.E. and were analyzed using a one-way analysis of variance with repeated measures and Scheffe's *F* test or a paired *t*-test (Snedecor and Cochran, 1980). A *P* value of < 0.05 was used as the criterion for statistical significance.

3. Results

3.1. Responses to angiotensin I, II, IV, and angiotensin I-(3–10)

Under constant-flow conditions, injections of angiotensin I, II, IV, and angiotensin I-(3–10) into the mesenteric arterial perfusion circuit caused dose-related increases in mesenteric arterial perfusion pressure (Fig. 1). Responses to the angiotensin peptides were reproducible with respect to time when injections were repeated on a 10–20 min basis (data not shown). When doses of the four angiotensin peptides are expressed on a nmol basis, the dose-response curves for the intra-arterial injections of angiotensin I and II were very similar (Fig. 1A). The dose-response curves for the intra-arterial injections of angiotensin I-(3–10) and angiotensin IV were also very similar (Fig. 1B). In terms of relative vasoconstrictor activity in the mesenteric vascular bed, angiotensin II was approximately 300 times more potent than angiotensin IV when doses are compared on a nmol basis (Fig. 1).

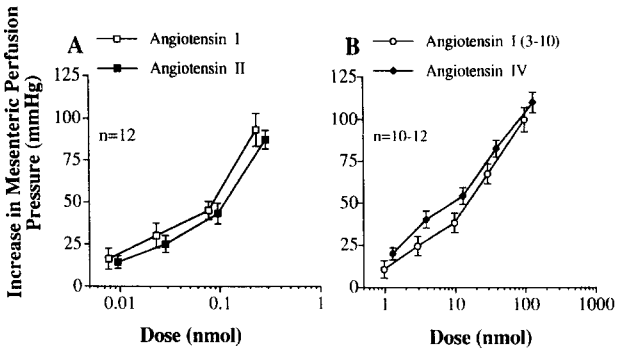

Fig. 1. Dose-response curves comparing increases in perfusion pressure in response to angiotensin I and II (A) and angiotensin I-(3–10) and angiotensin IV (B) in the mesenteric vascular bed of the cat. The peptides were injected directly into the mesenteric arterial perfusion circuit. *n* indicates number of animals.

The time-course for the increase in mesenteric arterial perfusion pressure in response to angiotensin I, II, angiotensin I-(3–10), and angiotensin IV, and the effect of a time-delay coil on the inlet side of the perfusion circuit are displayed in Figs. 2 and 3. The time of onset and for the peak increase in perfusion pressure in response to injections of angiotensin I and II at 0.3 μg into the mesenteric perfusion circuit was similar, whereas the duration of the response to the precursor was longer when compared with the duration of the response to angiotensin II (Fig. 2). The presence of a time-delay coil on the inlet side of the perfusion circuit had no effect on the amplitude or the time-course of the pressor response to angiotensin I or II (Fig. 2).

In a manner similar to that observed with angiotensin I and II, the time of onset and the time for attainment of the peak increase in perfusion pressure were similar when angiotensin I-(3–10) and angiotensin IV were injected in a dose of 30 μg into the perfusion circuit, whereas the duration of the response to the precursor was longer than the duration of the response to angiotensin IV (Fig. 3). The presence of the time-delay coil on the inlet side of the perfusion circuit had no significant effect on the amplitude or duration of the pressor response to angiotensin I-(3–10) or angiotensin IV (Fig. 3).

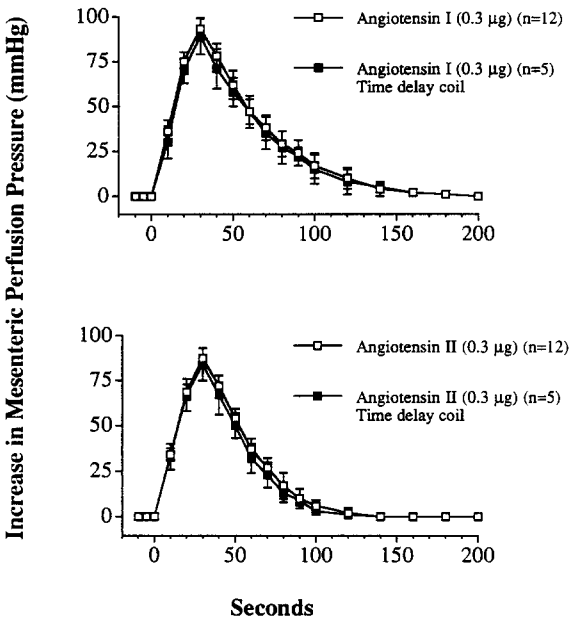

Fig. 2. Time-course of the increase in mesenteric arterial perfusion pressure in response to angiontensin I and II when the standard perfusion circuit was used and when a 30–60 s time-delay coil was placed on the inlet side of the perfusion circuit. The peptides were injected at time zero, and *n* indicates number of animals.

3.2. Effects of enalaprilat

The effects of the angiotensin converting enzyme inhibitor, enalaprilat, on pressor responses to angiotensin I and angiotensin I-(3–10) were investigated, and these data are summarized in Fig. 4. Following administration of enalaprilat in a dose of 4 mg/kg i.v., the increases in mesenteric arterial perfusion pressure in response to angiotensin I and angiotensin I-(3–10) were reduced significantly (Fig. 4). Following administration of the angiotensin converting enzyme inhibitor, the increases in mesenteric arterial perfusion pressure in response to angiotensin II and IV were not altered, whereas enalaprilat significantly increased the mesenteric vasodilator response to bradykinin (Fig. 4). The angiotensin converting enzyme inhibitor had no significant effect on the decrease in mesenteric arterial perfusion pressure in response to injections of the nitric oxide donor, *S*-nitroso-*N*-acetylpenicillamine (Fig. 4). The administration of enalaprilat in a dose of 4 mg/kg i.v. caused a significant decrease in systemic arterial and mesenteric arterial perfusion pressure (data not shown).

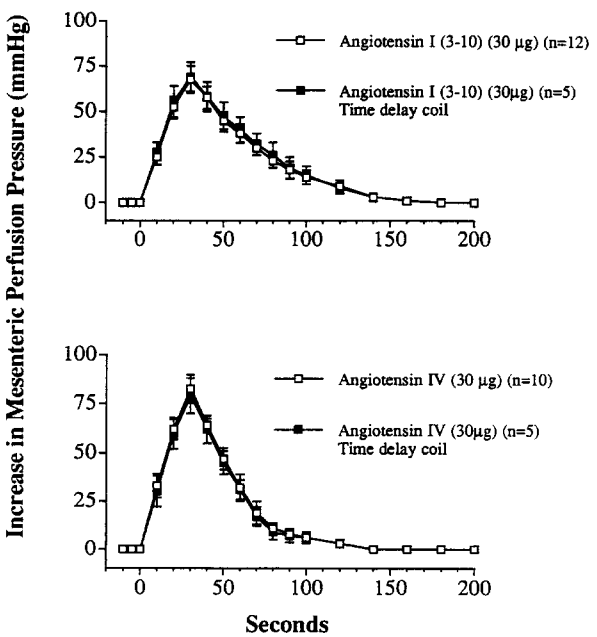

Fig. 3. Time-course of the increase in mesenteric arterial perfusion pressure in response to angiotensin I-(3–10) and angiotensin IV when the standard perfusion circuit was used and when a 30–60 s time-delay coil was placed on the inlet side of the perfusion circuit. The peptides were injected at time zero, and *n* indicates number of animals.

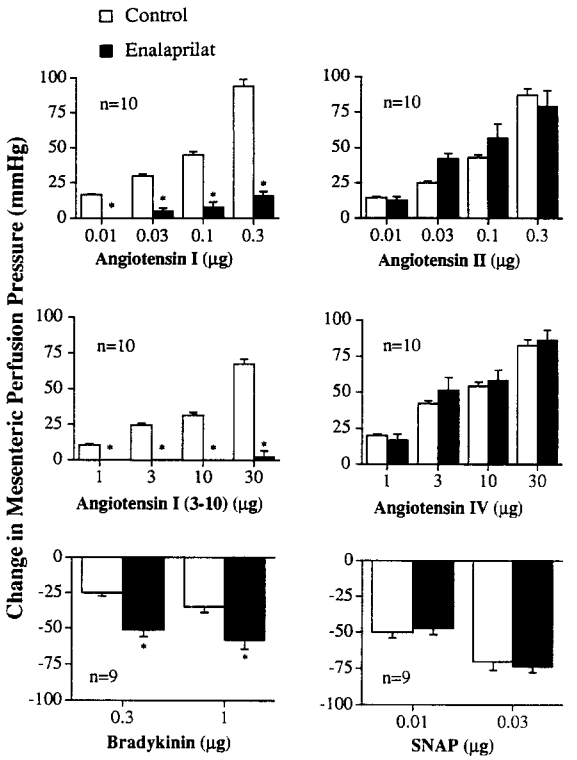

Fig. 4. Effect of the angiotensin converting enzyme inhibitor, enalaprilat, in doses of 4 mg/kg i.v. on responses to angiotensin I, II, IV, angiotensin I-(3–10), bradykinin, and *S*-nitroso-*N*-acetylpenicillamine (SNAP) in the mesenteric vascular bed of the cat. Responses were compared before and beginning 10 min after the administration of enalaprilat. *n* indicates number of experiments, and the asterisk indicates that responses are significantly different than control.

3.3. Effects of DuP532 and PD123,319 on responses to angiotensin IV

The effects of the angiotensin AT_1 receptor antagonist DuP532 on mesenteric vasoconstrictor responses to angiotensin II and IV were investigated, and these data are summarized in Fig. 5. Following administration of DuP532 in a dose of 100 μg/kg i.v., increases in mesenteric arterial perfusion pressure in response to angiotensin I and

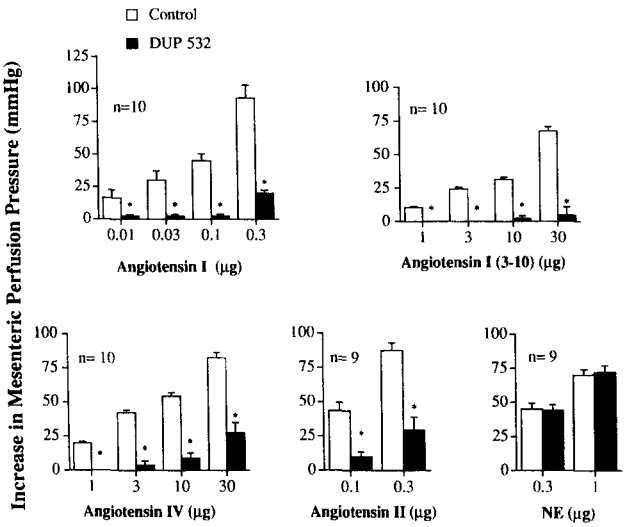

Fig. 5. Influence of the angiotensin AT_1 receptor antagonist, DuP532 (100 μg/kg i.v.), on responses to angiontensin I, II, IV, angiotensin I-(3–10), and norepinephrine (NE) in the mesenteric vascular bed of the cat. Responses were compared before and beginning 10 min after administration of the receptor antagonist. *n* indicates number of experiments, and the asterisk indicates that responses are significantly different than control.

angiotensin I-(3–10) were reduced significantly (Fig. 5). The angiotensin AT_1 receptor antagonist also significantly decreased pressor responses to angiotensin II and angiotensin IV without altering the pressor response to norepinephrine (Fig. 5). DuP532 had no significant effect on baseline systemic arterial pressure or on mesenteric arterial perfusion pressure when administered in a dose of 0.1 mg/kg i.v. (data not shown).

The effects of the angiotensin AT_2 receptor antagonist,

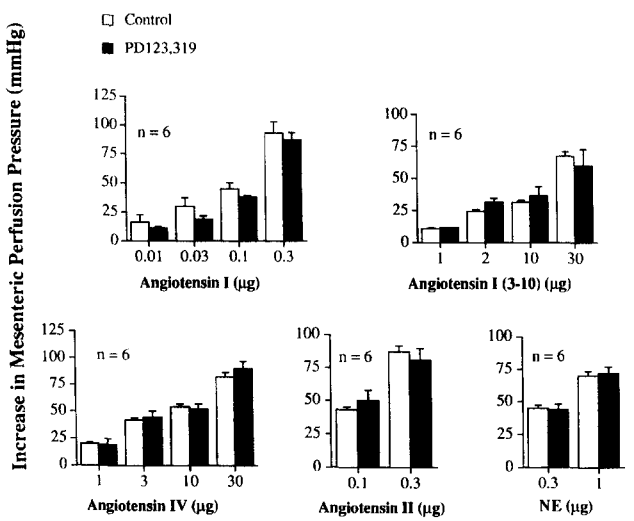

Fig. 6. Influence of the angiotensin AT_2 receptor antagonist, PD123,319 (5–10 mg/kg i.v.), on responses to angiotensin I, II, IV, angiotensin I-(3–10), and norepinephrine (NE) in the mesenteric vascular bed of the cat. Responses were compared before and beginning 10 min after administration of PD 123,319. *n* indicates number of experiments, and the asterisk indicates that the response is significantly different than control.

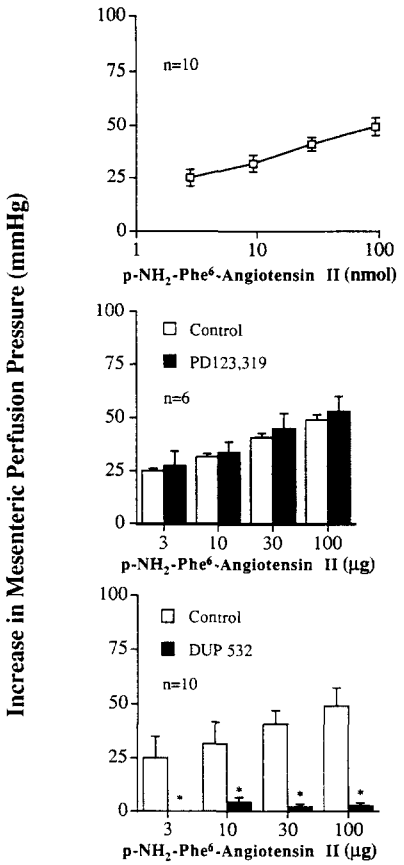

Fig. 7. Effect of the putative angiotensin AT_2 receptor agonist, *p*-aminophenylalamine^6 (*p*-NH_2-Phe^6)-angiotensin II, on mesenteric arterial perfusion pressure and the influence of PD123,319 (5–10 mg/kg i.v.) and DuP532 (100 μg/kg i.v.) on increases in mesenteric arterial perfusion pressure in response to *p*-NH_2-Phe^6-angiotensin II. Responses were compared before and beginning 10 min after administration of the receptor antagonists. *n* indicates number of animals, and the asterisk indicates that the response is significantly different than control.

PD123,319, on pressor responses to the angiotensin peptides were investigated, and these data are summarized in Fig. 6. Following administration of PD123,319 in a dose of 5–10 mg/kg i.v., increases in mesenteric arterial perfusion pressure in response to angiotensin I, II, IV, angiotensin I-(3–10), and to norepinephrine were not altered (Fig. 6). PD123,319, when injected in a dose of 5–10 mg/kg i.v., had no consistent effect on systemic arterial or mesenteric arterial perfusion pressure (data not shown).

In an additional series of experiments, vasoconstrictor responses to the angiotensin peptides were compared before and after administration of the putative AT_2 receptor antagonist, PD123,319, in a dose of 20 mg/kg i.v. in 3 cats, and these data are summarized in Table 1. Systemic arterial and mesenteric perfusion prssures (110 ± 5 and 121 ± 8, respectively) were not significantly altered 10 min after administration of PD123,319 in a dose of 20 mg/kg i.v. (112 ± 6 and 126 ± 7, respectively). The putative AT_2 antagonist was without significant effect on vasoconstrictor responses to *p*-aminophenylalanine^6-

Table 1
Influence of PD123,319 (20 mg/kg i.v.) on changes in mesenteric arterial perfusion pressure (mm Hg) in the cat

$n = 3$	Control	PD123,319
Angiotensin I		
0.03 μg	35±3	28±8
0.1 μg	45±5	48±5
0.3 μg	93±6	85±5
Angiotensin II		
0.03 μg	23±5	28±8
0.1 μg	43±3	45±5
0.3 μg	92±5	88±7
Angiotensin I-(3–10)		
3 μg	23±5	33±8
10 μg	35±5	43±8
30 μg	73±8	80±10
Angiotensin IV		
3 μg	38±3	35±5
10 μg	63±3	60±3
30 μg	80±7	72±10
p-Aminophenylalanine^6-angiotensin II		
10 μg	33±3	38±8
30 μg	38±8	43±5
100 μg	55±5	53±8
Bradykinin		
0.3 μg	−20±3	−25±6
1 μg	−45±5	−50±5
Norepinephrine		
0.3 μg	38±8	33±3
1 μg	65±5	63±5

angiotensin II when compared to control in the mesenteric vascular bed of the cat (Table 1). Increases in mesenteric arterial perfusion pressure in response to angiotensins I, II, and IV were not changed by PD123,319 in a dose of 20 mg/kg i.v. (Table 1). In addition, vasodilator responses to bradykinin and vasoconstrictor responses to norepinephrine were not significantly changed following administration of PD123,319 in a dose of 20 mg/kg i.v. (Table 1).

3.4. Responses to p-aminophenylalanine^6-angiotensin II

p-Aminophenylalanine^6-angiotensin II is a putative angiotensin AT_2 receptor agonist, and injections of the peptide in doses of 3–100 μg caused dose-related increases in mesenteric arterial perfusion pressure (Fig. 7). Increases in mesenteric arterial perfusion pressure in response to *p*-aminophenylalanine^6-angiotensin II were not changed following administration of PD123,319 in a dose of 5–10 mg/kg i.v. (Fig. 7). Following administration of DuP532 (100 μg/kg i.v.), increases in mesenteric arterial perfusion pressure in response to *p*-aminophenylalanine^6-angiotensin II were decreased significantly (Fig. 7).

4. Discussion

The results of the present study show that angiotensin I and II produce similar increases in perfusion pressure in the mesenteric vascular bed of the cat. Angiotensin I-(3–10) and angiotensin IV also produced similar increases in mesenteric arterial perfusion pressure, and these peptides were approximately 300-fold less potent than angiotensin I or II when doses are expressed on a nmol basis. The increases in perfusion pressure in response to angiotensin I and to angiotensin I-(3–10) were reduced significantly by enalaprilat, whereas the angiotensin converting enzyme inhibitor had no effect on responses to angiotensin II and IV and enhanced the vasodilator response to bradykinin. These results suggest that angiotensin I and angiotensin I-(3–10) are rapidly and efficiently converted into active peptides by an angiotensin converting enzyme-dependent pathway, and that angiotensin IV has significant vasoconstrictor activity in the mesenteric vascular bed of the cat. The rapidity and similarity in the time-course of the increase in perfusion pressure in response to local injections of angiotensin I and angiotensin I-(3–10) when compared with angiotensin II and IV and the absence of an effect of a time-delay coil on the pressor response suggest that the substrates are rapidly and efficiently converted into active peptides within the mesenteric vascular bed, presumably at or near the site of action of the peptides.

The site of action for angiotensin I has not been determined in the mesenteric vascular bed of the cat but has been shown by direct microscopic examination to be small arteries in the cremasteric vascular bed of the rat (Vicaut and Hou, 1993). The observation that responses to angiotensin I and II are similar in time-course and in magnitude suggests that conversion of precursor into active peptide is comparable to the observed in the hindlimb of the cat and is greater than the 19-36% conversion that has been reported to occur in the peripheral vascular bed of the dog (Franklin et al., 1970; DiSalvo et al., 1971; Aiken and Vane, 1972; Garrison et al., 1995). The reason for the efficient conversion of angiotensin I to II in the feline mesenteric vascular bed when compared with other vascular beds in the dog is unknown but suggests that angiotensin converting enzyme activity is high in upstream vessels and that angiotensin I could play a role in the regulation of vascular resistance in the mesenteric circulation of the cat.

The angiotensin receptor subtype mediating the increase in mesenteric arterial perfusion pressure in response to the angiotensin peptides was investigated, and pressor responses to angiotensin II and to angiotensin IV were decreased by DuP532 in a dose that had no significant effect on the response to norepinephrine. Pressor responses to angiotensin I and to angiotensin I-(3–10) were also attenuated by DuP532. The results of studies with DuP532 indicate that vasoconstrictor responses to angiotensin II and IV and to their precursors are mediated by the activa-

tion of angiotensin AT_1 receptors. The angiotensin AT_1 receptor antagonist had little, if any, effect on baseline systemic arterial or mesenteric perfusion pressure whereas enalaprilat significantly decreased these pressures. The explanation for difference in effect of enalaprilat and the angiotensin AT_1 receptor blocker may involve an effect of the angiotensin converting enzyme inhibitor to increase levels of bradykinin and vasodilator prostaglandins. The role of angiotensin AT_2 receptor activation in the mediation of responses to angiotensin II and IV was also investigated, and PD123,319 in a dose of 5–20 mg/kg i.v. had no significant effect on the response to angiotensin II or IV or their substrates and had no significant effect on the pressor response to norepinephrine. The efficacy of the angiotensin AT_2 receptor blockage with PD123,319 is difficult to evaluate without the availablity of studies with an angiotensin AT_2 receptor agonist. *p*-Aminophenylalanine^6-angiotensin II has been reported to be an agonist with high affinity for the angiotensin AT_2 receptor, which increases thirst and sodium appetite in rats; and in the present study in the mesenteric vascular bed of the cat, this agent produced dose-related increases in mesenteric arterial perfusion pressure (Speth and Kim, 1990; Cooney and Fitzsimmons, 1993). However, in the present study the increases in perfusion pressure in response to *p*-aminophenylalanine^6-angiotensin II were significantly attenuated by DuP532, whereas PD123,319 had no significant effect on the response to this putative angiotensin mesenteric receptor agonist. These data suggest that *p*-aminophenylalanine^6-angiotensin II increases mesenteric vascular resistance by activating angiotensin AT_1 receptors as has been previously suggested in studies on thirst and sodium appetite in the conscious rat (Cooney and Fitzsimmons, 1993) and in the hindlimb vascular bed of the cat (Garrison et al., 1995).

The observation that vasoconstrictor responses to angiotensin II and IV and to their substrates are reduced by DuP532 but not by PD123,319 may be interpreted to suggest that vasoconstrictor responses to the angiotensin peptides are mediated by the activation of angiotensin AT_1 receptors, and that angiotensin AT_2 receptors play little if any role in mediating or in modulating responses to these peptides in the mesenteric vascular bed of the cat. The inability of PD123,319 to alter responses to the angiotensin peptides in the mesenteric vascular bed may be related to the dose of the angiotensin AT_2 antagonist used or to an absence of angiotensin AT_2 receptors in resistance vessels in the mesenteric vascular bed. It would, therefore, be useful to assess the presence of angiotensin AT_2 receptors by binding studies or by measuring the messenger RNA for these receptors in mesenteric resistance vessels. The absence of an effect of PD123,319 on responses to angiotensin II or IV without an internal control response to establish efficacy of the angiotensin AT_2 receptor blockage makes the data with PD123,319 somewhat difficult to interpret, although studies in the rat suggest that doses of PD123,319 in the range of 10 mg/kg i.v. were capable of modifying arterial pressure responses to angiotensin II and III (Scheuer and Perrone, 1993).

The observation that PD123,319 in doses as high as 20 mg/kg i.v. had no significant effect on vasoconstrictor responses to angiotensin I, II, II, or angiotensin I-(3–10) suggests that angiotensin II AT_2 receptors play little, if any, role in the mediation or modulation of responses to the angiotensin peptides in the mesenteric vascular bed of the cat.

Novel non-AT_1/AT_2 binding sites for angiotensin IV have been shown to be present in a number of tissues, including cultured vascular smooth muscle and endothelial cells (Harding et al., 1992; Hall et al., 1993; Hanesworth et al., 1993; Pörst et al., 1994). Angiotensin IV has been reported to have modest pressor activity in normal human subjects (Semple et al., 1976) and to increase mean arterial pressure and reduce renal and mesenteric blood flow in the conscious rat (Gardiner et al., 1993). The observation that vasoconstrictor responses to the hexapeptide are blocked by losartan is consistent with studies in the hindquarters and mesenteric vascular beds of the cat and provides support for the hypothesis that angiotensin IV has significant vasoconstrictor activty but that the peptide has an apparent low affinity for the angiotensin AT_1 receptor (Gardiner et al., 1993; Garrison et al., 1995). Although non-AT_1/AT_2 binding sites for angiotensin IV are present in cultured endothelial and vascular smooth muscle cells (Harding et al., 1992; Hall et al., 1993; Hanesworth et al., 1993; Pörst et al., 1994), the presence or role of non-AT_1/AT_2 angiotensin IV binding sites has not been established in the mesenteric vascular bed of the cat, and the present data with DuP532 provide support for the hypothesis that responses to the hexapeptide are mediated by angiotensin AT_1 receptors.

Angiotensin IV has been reported to increase cortical blood flow when infused into the rat kidney and dilates rabbit cerebral arterioles when administerd with L-arginine, the precursor for nitric oxide (Haberl et al., 1991; Coleman et al., 1993). However, in the present study, vasodilator responses to angiotensin IV are not seen in the mesenteric vascular bed, and these data are consistent with results obtained in the hindlimb vascular bed of the cat (Garrison et al., 1995) and the renal and mesenteric vascular bed of the rat (Gardiner et al., 1993). The explanation for the difference in results in peripheral vascular beds in the rat and cat when compared with results obtained in the cerebral circulation in the rabbit is uncertain but may reflect differences in species or experimental preparation utilized (Haberl et al., 1991).

Results of the present study indicate that angiotensin I and angiotensin I-(3–10) are rapidly and efficiently converted into active peptides by an angiotensin converting enzyme-dependent pathway at or near the site of action of these peptides within the mesenteric vascular bed. These data may be interpreted to suggest that substrates, such as

angiotensin I or angiotensin I-(3–10), could influence vascular resistance and have a local regulatory function in the mesenteric circulation if sufficient amounts were formed within upstream segments in the bed.

In summary, the results of the present study show that angiotensin I and angiotensin I-(3–10) are rapidly and efficiently converted into active peptides within the mesenteric vascular bed of the cat. The vasoconstrictor responses to the precursors are reduced by enalaprilat, suggesting that the substrates are converted into active peptides by an angiotensin converting enzyme-dependent pathway. Pressor responses to the precursors and to angiotensin II and IV are attenuated by DuP532 but are not altered by PD123,319, suggesting that responses to these peptides are mediated by the activation of angiotensin AT_1 receptors and that angiotensin AT_2 receptors play little, if any, role in mediating or modulating these responses. *p*-Aminophenylalanine^6-angiotensin II produced dose-dependent increases in mesenteric vascular resistance, and responses to this peptide were reduced by DuP532, suggesting that *p*-aminophenylalanine^6-angiotensin II stimulates angiotensin AT_1 receptors. The results of the present study suggest that angiotensin I-(3–10) and angiotensin I could act locally to regulate vasomotor tone within the mesenteric vascular bed of the cat if formed in sufficient quantities.

References

Ahmad, S. and P.E. Ward, 1990, Role of aminopeptidase activity in the regulation of the pressor activity of circulating angiotensins, J. Pharmacol. Exp. Ther. 252, 643.

Aiken, J.W. and J.R. Vane, 1972, Inhibition of converting enzyme of the renin angiotensin system in kidneys and hindlegs of dogs, Circ. Res. 30, 263.

Bernier, S.G., A. Fournier and G. Guillemette, 1994, A specific binding site recognizing a fragment of angiotensin II in bovine adrenal cortex membranes, Eur. J. Pharmacol. 271, 55.

Blair-West, J.R., J.P. Coghlan, D.A. Denton, J.W. Funder, B.A. Scoggins and R.D. Wright, 1971, The effect of the heptapeptide (2–8) and hexapeptide (3–8) fragments of angiotensin II on aldosterone secretion, J. Clin. Endocrinol. Metab. 32, 575.

Blankley, C.J., J.C. Hodges, S.R. Klutchko, R.J. Himmelsbach, A. Chucholowski, C.J. Connolly, S.J. Neergaard, M.S. van Nieuwenhze, A. Sebastian and J. Quin III, 1991, Synthesis and structure-activity relationship of a novel series of non-peptide angiotensin II receptor binding inhibitors specific for the AT_2 subtype, J. Med. Chem. 34, 3248.

Bottari, S.P., M. de Gasparo, U.M. Steckelings and N.R. Levens, 1993, Angiotensin II receptor subtypes: characterization, signaling mechanisms, and possible physiological implications, Front. Neuroendocrinol. 14, 123.

Cheng, D.Y., B.J. DeWitt, E.L. Dent, B.D. Nossaman and P.J. Kadowitz, 1994, Analysis of responses to angiotensin IV in the pulmonary vascular bed of the cat, Eur. J. Pharmacol. 261, 223.

Chiu, A.T., J.W. Ryan, J.M. Stewart and F.E. Dorer, 1976, Formation of angiotensin III by angiotensin converting enzyme, Biochem. J. 155, 189.

Coleman, J.K.M., L. Keebs, B. Ong, J.M. Hanesworth, K.A. Lawrence, M. Sardinia, J.W. Harding and J.W. Wright, 1993, Changes in renal blood flow due to intraarterial infusions of angiotensin II (3–8) [AIV] and AIV analogs, FASEB J. 7, A437.

Cooney, A.S. and J.T. Fitzsimmons, 1993, The effect of the putative AT_2 agonist, *p*-aminophenylalanine^6 angiotensin II on thirst and sodium appetite in rats, Exp. Physiol. 78, 767.

DiSalvo J., C.b. Montefusco, 1971, Conversion of angiotensin I to angiotensin II in the canine mesenteric circulation, Am. J. Physiol. 221, 1576.

Franklin, W.G., M.J. Peach and J.P. Gilmore, 1970, Evidence for the renal conversion of angiotensin I in the dog, Circ. Res. 27, 321.

Garrison, E.A., J.A. Santiago, S.Y. Osei and P.J. Kadowitz, 1995, Analysis of responses to angiotensin peptides in the hindquarters vascular bed of the cat, Am. J. Physiol. 268 (Heart Circ. Physiol. 37), H2418.

Gardiner, S.M., P.A. Kemp, J.E. March and T. Bennett, 1993, Regional hemodynamic effects of angiotensin II (3–8) in conscious rats, Br. J. Pharmacol. 110, 159.

Haberl, R.L., R. Decker and K.M. Einhaupl, 1991, Angiotensin degradation products mediate endothelium-dependent dilation of rabbit brain arterioles, Circ. Res. 68, 1621.

Hall, K.L., J.M. Hanesworth, A.E. Ball, G.P. Felgenhauer, H.L. Hosick and J.W. Harding, 1993, Identification and characterization of a novel binding site in cultured vascular smooth muscle cells that is specific for the hexapeptide (3–8) fragment of angiotensin II, angiotensin IV, Regul. Peptides 44, 225.

Hanesworth, J.M., M.F. Sardinia, L.T. Krebs, K.L. Hall and J.W. Harding, 1993, Elucidation of a specific binding site for angiotensin II (3–8), angiotensin IV, in mammalian heart membranes, J. Pharmacol. Exp. Ther. 266, 1036.

Harding, J.W., V.I. Cook, A.V. Miller-Wing, J.M. Hanesworth, M.F. Sardinia, K.L. Hall, J.W. Stobb, G.N. Swanson, J.K.M. Coleman, J.W. Wright and E.C. Harding, 1992, Identification of an AII(3-8) [AIV] binding site in guinea pig hippocampus, Brain Res. 583, 340.

Kono, T., F. Ikeda, A. Tamiguchi, H. Imura, F. Oseko, M. Yoshioka and M.C. Khosla, 1985, Responses of patients with Bartter's syndrome to angiotensin III and angiotensin II (3–8)-hexapeptide, Acta Endocrinol. 109, 240.

Nossaman, B.D., C.J. Feng, J. Wand and P.J. Kadowitz, 1994, Analysis of angiotensin I, II, and III in the pulmonary vascular bed of the rat, Am. J. Physiol. 266, L389.

Osei, S.Y., R.S. Ahima, R.K. Minkes, J.P. Weaver, M.C. Khosla and P.J. Kadowitz, 1993, Differential responses to angiotensin-(1–7) in the feline mesenteric and hindquarters vascular beds, Eur. J. Pharmacol. 234, 35.

Peach, M.J., 1977, Renin-angiotensin system: biochemistry and mechanisms of action, Physiol. Rev. 57, 313.

Pörst, I., A.T. Bara, R. Busse and M. Hecker, 1994, Release of nitric oxide by angiotensin-(1–7) from porcine coronary endothelium: implications for a novel angiotensin receptor, Br. J. Pharmacol. 111, 652.

Regoli, D., W.K. Park and F. Rioux, 1974, Pharmacology of angiotensin, Pharmacol. Rev. 26, 69.

Scheuer, D.A. and M.H. Perrone, 1993, Angiotensin type 2 receptors mediate depressor phase of biphasic pressure response to angiotensin, Am. J. Physiol. 264, R917.

Semple, P.F., A.S. Boyd, P.M. Dawes and J.J. Morton, 1976, Angiotensin II and its heptapeptide (2-8), hexapeptide (3–8), and pentapeptide (4–8) metabolites in arterial and venous blood of man, Circ. Res. 39, 671.

Snedecor, G.W. and W.G. Cochran, 1980, Statistical Methods, Iowa State University Press, Ames.

Speth, R.C. and K.H. Kim, 1990, Discrimination of two angiotensin II receptor subtypes with a selective agonist analogue of angiotensin II, *p*-aminophenylalanine^6 angiotensin II, Biochem. Biophys. Res. Commun. 169, 997.

Swanson, G.N., J.M. Hanesworth, M.F. Sardinia, J.K.M. Coleman, J.W. Wright, K.L. Hall, A.V. Miller-Wing, J.W. Stobb, V.I. Cook, E.C.

Harding and J.W. Harding, 1992, Discovery of a distinct binding site for angiotensin II(3–8), a putative angiotensin IV receptor, Regul. Pept. 40, 409.

Timmermans, P.B.M.W.M., P.C. Wong, A.T. Chiu, W.F. Herblin, P. Benfield, D.J. Carini, R.J. Lee, R.R. Wexler, J.M. Saye and R.D. Smith, 1993, Angiotensin II receptors and angiotensin II receptor antagonists, Pharmacol. Rev. 45, 105.

Vicaut, E. and X. Hou, 1993, Arteriolar constriction and local renin = angiotensin system in rat microcirculation, Hypertension 21, 491.

Ward, P.E., 1984, Immunoelectrophoretic analysis of vascular membrane-bound angiotensin I converting enzyme, aminopeptidase M, and dipeptidal (amino) peptide IV, Biomed. Pharmacol. 33, 3183.